

## The effect of insulin infusion and food intake on muscle protein synthesis in postabsorptive rats

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1. Insulin was infused into young male rats in the postabsorptive state. Rates of protein synthesis in skeletal muscle were determined during the final 10 min of infusion from the incorporation of label into protein after intravenous injection of a massive dose of [<sup>3</sup>H]phenylalanine. Rates of synthesis were not altered during the first 10 min of insulin infusion, but were increased significantly between 10 and 60 min. 2. Rats were infused with different amounts of insulin for 30 min. When concentrations were increased from 10 to 40  $\mu$ units/ml of plasma there was no change in muscle protein synthesis, but concentrations higher than 70  $\mu$ units/ml caused a significant stimulation. Concentrations below 10  $\mu$ units/ml, obtained by infusion of anti-insulin serum, did not depress synthesis below that found in the postabsorptive rat. 3. Infusion of glucose for 30 or 60 min led to an increase in plasma insulin to 40  $\mu$ units/ml, but this also failed to stimulate muscle protein synthesis. 4. Rates of synthesis in postabsorptive rats, even when stimulated maximally by insulin, were not so high as those in fed rats or in postabsorptive rats refed for 60 min. However, in fed and refed rats insulin concentrations were below that required to stimulate synthesis in postabsorptive animals. Despite this, infusion of large amounts of insulin into fed rats did not increase synthesis further. 5. The sensitivity of plasma glucose to insulin infusion was different from that of protein synthesis. A decrease in glucose concentration preceded the increase in synthesis and occurred at lower insulin concentrations. 6. It is concluded that changes in circulating insulin may have been partly responsible for the increase in muscle protein synthesis brought about by feeding, but that other factors must also play a part.

A role for insulin in the regulation of protein synthesis in skeletal muscle has been known for many years (Manchester, 1970). Diabetic animals suffer loss of body protein and a failure of protein synthesis in various tissues, particularly muscle. This has been demonstrated both *in vivo* (Pain & Garlick, 1974) and *in vitro* (Flaim *et al.*, 1980). In both of these studies a stimulation of synthesis was achieved by administration of insulin. Studies *in vitro* on perfused or incubated muscles from non-diabetic rats have also demonstrated a role for insulin in maintaining protein synthesis (e.g. Fulks *et al.*, 1975; Jefferson *et al.*, 1974). In the perfused hemicorpus from fed rats there was a decline in protein synthesis during the first hour, which was attributed to a block in the rate of peptide-chain initiation, and this could be prevented by insulin (Jefferson *et al.*, 1974). In preparations from starved rats the rate of synthesis was lower, but on addition

of insulin it was increased to a value similar to that for fed rats (Li *et al.*, 1979; Preedy *et al.*, 1979).

Measurements *in vivo* have also shown that muscle protein synthesis is less in starved than in fed rats. Since the concentration of insulin in starved animals is also lower, it is possible therefore that the response of protein synthesis to feeding and starvation is partly or wholly brought about by the changes in insulin. It is important to recognize that some of the effect may also result from changes in other hormones and metabolites. For example, amino acids, particularly leucine, have been shown to influence the rate of protein synthesis in muscles *in vitro* (e.g. Fulks *et al.*, 1975; Li & Jefferson, 1978), although this effect has not been confirmed by experiments *in vivo* (McNurlan *et al.*, 1982).

We have therefore investigated the effects of insulin infusion on muscle protein synthesis *in vivo* in postabsorptive rats. The aim was to discover how

much of the effect of feeding could be attributed to insulin at normal physiological concentrations. Most of the earlier experiments *in vitro* used very high concentrations of the hormone (e.g. 25 munits/ml), and, although effects have been noted at concentrations lower than 100  $\mu$ units/ml (Jefferson *et al.*, 1977; Frayn & Maycock, 1979), it is important to know how this relates to the concentration that is generally found in a fed rat.

## Methods

### Animals

Male Wistar rats were purchased from Charles River (Margate, Kent, U.K.) at a weight of 85 g and were maintained in a temperature-controlled animal house with the lights on between 08:00 and 20:00 h each day. They were given a commercial pellet diet containing 23% crude protein (Oxoid, Basingstoke, Hants., U.K.) *ad libitum* for 6–8 days before experimentation, by which time they had reached about 130 g. For measurements of protein synthesis, a batch of animals (identified by a single experiment number) were divided between 2 (or sometimes 3) consecutive days, and groups were also distributed uniformly among these days. All infusions and injections were performed between 10:00 and 13:00 h. Food was removed from rats designated 'postabsorptive' at 23:00 h the previous night.

### Experimental procedure

Immediately before infusion each rat was immobilized in a towel, and a 26-gauge needle attached to a length of polypropylene tubing was inserted into a lateral tail vein as described previously (Garlick *et al.*, 1975). Monocomponent insulin (Actrapid and diluent, supplied by Novo, Basingstoke, Hants., U.K.) was infused at the appropriate rate for various periods up to 1 h. The same procedure was used for infusing insulin diluent (controls), glucose [10% (w/v) dextrose; Travenol Laboratories, Thetford, Norfolk, U.K.] or guinea-pig serum [anti-(human insulin) serum; Wellcome Reagents, Beckenham, Kent, U.K.; or serum obtained locally from normal guinea pigs].

At 10 min before the end of the infusion about 25  $\mu$ Ci (150  $\mu$ mol) of [4-<sup>3</sup>H]phenylalanine was injected into the infusion cannula for measurement of the rate of protein synthesis (Garlick *et al.*, 1980), and the infusion continued until death. After precisely 10 min the rat was decapitated, blood was collected and the gastrocnemius muscle was rapidly removed and frozen as described previously (Garlick *et al.*, 1980).

### Chemical determinations

Muscles were homogenized and prepared for measurement of the specific radioactivity of free and

protein-bound phenylalanine and the concentrations of RNA and protein, as described previously (Garlick *et al.*, 1980; McNurlan *et al.*, 1982). Blood was centrifuged and the concentration of insulin in plasma measured by radioimmunoassay (Herbert *et al.*, 1965), with human insulin as a standard. Plasma glucose concentration was measured by the glucose oxidase method with a kit from Boehringer Corp. (London) Ltd., London W.5, U.K.

### Calculation of rates of protein synthesis

The fractional rate of protein synthesis ( $k_s$ , expressed as %/day) was calculated from the following formula (McNurlan *et al.*, 1979; Garlick *et al.*, 1980):

$$k_s = \frac{S_b \times 100}{S_f \times t}$$

where  $S_b$  and  $S_f$  are the specific radioactivities of protein-bound and free phenylalanine in the muscle, and  $t$  is the time of incorporation of label (i.e. 10 min plus 0.5 min for removal of the muscle after killing) expressed in days.

This formula assumes that the specific radioactivity of free phenylalanine is constant during the 10 min incorporation period. We have previously shown that in practice there is a slow linear decline (Garlick *et al.*, 1980), but in the present study of muscle it was reasonable to neglect this because the decline is always very small. In preliminary experiments we have shown that the mean specific radioactivity over the 10 min labelling period is less than 5% higher than the value at 10 min and that the difference is not influenced by insulin injection. A second assumption is that the free phenylalanine in the muscle can be used to represent the specific radioactivity at the site of protein synthesis. The large dose of labelled amino acid was given so that all pools of free phenylalanine, including that of phenylalanyl-tRNA, would attain similar specific radioactivities. Whereas the ability of large amounts of amino acid to flood the pools in this way has been questioned in studies on cells in culture (Hildebran *et al.*, 1981; Hammer & Rannels, 1981), the evidence from experiments on whole tissues suggests that flooding can indeed be achieved. In liver, heart and lung perfused with high concentrations of labelled amino acid the specific radioactivity of the tRNA has been shown to be close to that of the free amino acid in the perfusate and tissue (Khairallah & Mortimore, 1976; McKee *et al.*, 1978; Watkins & Rannels, 1980). When 150  $\mu$ mol of [<sup>3</sup>H]phenylalanine/100 g body wt. is injected, the specific radioactivities in the tissue and in the plasma do not differ by more than a few per cent, so our choice of the amino acid in the tissue to represent the precursor for protein synthesis is unlikely to result in any serious error.

**Statistics**

All results are presented as means  $\pm$  s.e.m., with numbers of observations in parentheses. Statistical significances of differences between groups within a single experiment were tested initially by analysis of variance, followed by Bonferroni's modified *t* test for multiple simultaneous comparisons, as described by Wallenstein *et al.* (1980). Logarithms of insulin values were taken before these tests were performed.

**Results**

Infusion of insulin into postabsorptive rats resulted in high concentrations of insulin in plasma within 10 min (Fig. 1). These were then maintained up to 1 h. Plasma glucose decreased very quickly during the first 20 min, but thereafter a nearly constant state of hypoglycaemia was maintained, at about 30% the concentration in the postabsorptive state. In spite of

the low plasma glucose concentrations, however, no animal showed overt signs of distress.

Rates of protein synthesis measured over 10 min intervals during insulin infusion are shown in Table 1. Values are expressed both as fractional rates ( $k_s$ , %/day) and as g of protein synthesized/day per g of RNA. The latter takes account of possible differences in the concentration of RNA, which would reflect differences in ribosome content, since a large proportion of tissue RNA is believed to be ribosomal (Henshaw *et al.*, 1971; Millward *et al.*, 1973). During the interval 0–10 min (Expt. 1) there was no discernible change in synthesis, but between 10 and 20 min there was an increase, which was statistically significant when expressed per g of RNA. During all periods between 10 and 60 min (Expts. 1 and 2) the rate of synthesis was significantly increased, when expressed either in terms of the RNA or as a fractional rate.

Table 2 shows the results of two experiments to demonstrate the effects of 30 min infusions of insulin at different rates. In Expt. 3 the infusion of insulin at 38 munits/h significantly increased the plasma insulin to 38  $\mu$ units/ml and significantly decreased the plasma glucose, but had no effect on the rate of protein synthesis. A higher rate of infusion, sufficient to raise the insulin concentration to 70  $\mu$ units/ml, caused a significant increase in protein synthesis, however, but it was not increased further by a still higher rate of infusion. In Expt. 4 there was evidence that the insulin had been adsorbed on to some part of the apparatus, since the two lowest rates of infusion gave lower concentrations in the plasma than expected. The lowest rate, 15 munits/h, did not alter the insulin and glucose concentrations nor the rates of protein synthesis, so this group acts as a second control. As with Expt. 3, low concentrations of insulin had no effect on protein synthesis, but significantly decreased the plasma glucose. Again, when the concentration of insulin was increased to about 70  $\mu$ units/ml there was a significant increase in

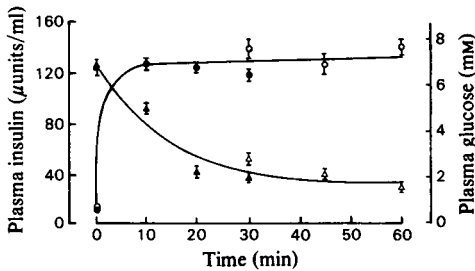


Fig. 1. Changes in concentrations of glucose ( $\Delta$ ,  $\blacktriangle$ ) and insulin ( $\circ$ ,  $\bullet$ ) in plasma of postabsorptive rats infused with insulin

Blood was collected from rats killed at the times shown. In Expt. 1 (closed symbols) rats were infused for 0, 10, 20 and 30 min, and in Expt. 2 (open symbols) for 0, 30, 45 and 60 min. For additional details see Table 1.

Table 1. Time course of response of muscle protein synthesis to insulin infusion in postabsorptive rats

Food was withdrawn from rats at 23:00h the previous night. Insulin was infused for the time period shown at a rate of 1.0 ml/h, 200 munits/ml. Control rats received no infusion. Protein-synthesis rates were measured during the last 10 min of infusion by injection of [ $^3$ H]phenylalanine into the infusion cannula. The infusion was then continued for the remaining 10 min before death. Significance of differences from control: \* $P < 0.05$ ; \*\* $P < 0.005$ ; \*\*\* $P < 0.001$ .

Expt. 1			Expt. 2		
Time (min)	$k_s$ (%/day)	Protein synthesis (g/day per g of RNA)	Time (min)	$k_s$ (%/day)	Protein synthesis (g/day per g of RNA)
Control (6)	10.4 $\pm$ 0.6	10.9 $\pm$ 0.7	Control (6)	10.2 $\pm$ 0.5	11.3 $\pm$ 0.6
10 (7)	9.6 $\pm$ 0.5	10.6 $\pm$ 0.4	30 (6)	14.0 $\pm$ 0.3***	14.6 $\pm$ 0.5**
20 (7)	12.1 $\pm$ 0.5	13.2 $\pm$ 0.6*	45 (5)	14.6 $\pm$ 0.6***	15.5 $\pm$ 0.5***
30 (7)	13.2 $\pm$ 0.4**	14.5 $\pm$ 0.6***	60 (6)	13.2 $\pm$ 0.8**	13.7 $\pm$ 0.6*

Table 2. *Effect of insulin concentration on protein synthesis in muscle of postabsorptive rats*

Rats were deprived of food from 23:00h the previous day. Insulin solutions were prepared at concentrations appropriate for the nominal infusion rates given in the Table. All rats received 30min infusions at a rate of 0.48 ml/h, and the rate of protein synthesis was measured during the last 10min. Significance of differences from control: \* $P < 0.05$ ; \*\* $P < 0.002$ .

Expt. no.	Insulin infusion (munits/h)	Plasma insulin ( $\mu$ units/ml)	Plasma glucose (mM)	$k_p$ (%/day)	Protein synthesis (g/day per g of RNA)
3	0 (6)	10 $\pm$ 1	6.1 $\pm$ 0.1	9.8 $\pm$ 0.7	10.5 $\pm$ 0.6
	38 (6)	38 $\pm$ 3**	4.5 $\pm$ 0.3**	10.5 $\pm$ 0.4	11.3 $\pm$ 0.5
	96 (7)	70 $\pm$ 5**	2.7 $\pm$ 0.2**	12.8 $\pm$ 0.7*	13.1 $\pm$ 0.6*
	152 (6)	107 $\pm$ 5**	2.4 $\pm$ 0.1**	12.7 $\pm$ 1.1*	12.8 $\pm$ 1.0
4	0 (6)	8 $\pm$ 1	6.9 $\pm$ 0.4	9.8 $\pm$ 0.3	10.6 $\pm$ 0.5
	15 (5)	7 $\pm$ 1	7.1 $\pm$ 0.4	10.2 $\pm$ 0.4	11.4 $\pm$ 0.4
	37 (5)	19 $\pm$ 1*	5.5 $\pm$ 0.4*	10.0 $\pm$ 0.3	11.1 $\pm$ 0.2
	87 (6)	69 $\pm$ 7**	3.2 $\pm$ 0.1**	11.3 $\pm$ 0.4*	12.8 $\pm$ 0.5**
	187 (6)	122 $\pm$ 7**	2.5 $\pm$ 0.3**	12.0 $\pm$ 0.5**	13.6 $\pm$ 0.3**

Table 3. *Effects of infusion of glucose on muscle protein synthesis in postabsorptive rats*

Rats were deprived of food from 23:00h the previous day. Glucose was infused at a rate of 0.48 g/h (2.4 ml/h) and protein synthesis was measured during the last 10min. These animals formed part of Expt. 4 (see Table 2). Significance of differences from control: \* $P < 0.001$ . There were no statistically significant differences between fractional rates of synthesis ( $k_p$ ) or synthesis per g of RNA.

Treatment	Plasma insulin ( $\mu$ units/ml)	Plasma glucose (mM)	$k_p$ (%/day)	Protein synthesis (g/day per g of RNA)
Control (6)	8 $\pm$ 1	6.9 $\pm$ 0.4	9.8 $\pm$ 0.3	10.6 $\pm$ 0.5
Glucose 30 min (6)	41 $\pm$ 4*	14.0 $\pm$ 0.3*	10.2 $\pm$ 0.3	11.1 $\pm$ 0.4
Glucose 60 min (5)	41 $\pm$ 7*	13.0 $\pm$ 0.5*	9.6 $\pm$ 0.4	10.7 $\pm$ 0.3

Table 4. *Effect of feeding and refeeding on protein synthesis in muscle*

Fed rats had access to food until injection of [ $^3$ H]phenylalanine; postabsorptive and refeed rats were deprived of food from 23:00h the previous day. Food was reintroduced to refeed rats 60min before [ $^3$ H]phenylalanine injection. These animals formed part of Expt. 2 (see Table 1). Significance of differences from postabsorptive, † $P < 0.001$ , and from fed, \* $P < 0.01$ .

Treatment	Plasma insulin ( $\mu$ units/ml)	Plasma glucose (mM)	$k_p$ (%/day)	Protein synthesis (g/day per g of RNA)
Postabsorptive (6)	13 $\pm$ 1	7.0 $\pm$ 0.3	10.2 $\pm$ 0.5	11.3 $\pm$ 0.6
Fed (6)	28 $\pm$ 3†	9.6 $\pm$ 0.2†	17.4 $\pm$ 0.4†	17.6 $\pm$ 0.4†
Refeed (6)	36 $\pm$ 3†	10.8 $\pm$ 0.2†*	14.6 $\pm$ 0.5†*	16.1 $\pm$ 0.6†

protein synthesis. There was a small further increase in synthesis when insulin rose to 122  $\mu$ units/ml.

Table 3 confirms that low concentrations of insulin (40  $\mu$ units/ml) had no effect on protein synthesis. The insulin was produced endogenously by infusion of glucose, but even when the infusion was extended to 60min there was no detectable change in synthesis.

The response to food intake is shown in Table 4. Animals that had been fed *ad libitum* until injection of [ $^3$ H]phenylalanine had higher concentrations of insulin and glucose in plasma than did postabsorptive rats. Rates of protein synthesis were also considerably higher. Since the ratio of RNA/protein

was slightly higher in fed animals (results not shown), the differences between fed and postabsorptive rats was greater for  $k_p$  than for synthesis per g of RNA. Refeeding of postabsorptive rats for 60min also resulted in higher concentrations of insulin and glucose in the plasma. Indeed, values were somewhat higher than in rats fed *ad libitum*. Protein synthesis was also increased, but rates were not so high as those in fed rats. The difference between fed and refeed rats was significant for  $k_p$ , but was less pronounced for synthesis per g of RNA, because refeeding for only 1h did not restore the RNA/protein ratio to its value for fed rats.

These measurements on fed and refeed rats were

performed as part of Expt. 2 (Table 1), which enables statistical comparisons to be made between the effects of food intake and of insulin infusion. The maximum effect of insulin on protein synthesis (45 min) was quite similar to that of refeeding, but the rate fell short of that in rats fed *ad libitum*. The difference in  $k_p$  between fed rats and those given insulin for 45 min was significant ( $P \approx 0.01$ ), but was not significant for synthesis per g of RNA ( $P \approx 0.1$ ). It should be noted, however, that the insulin concentration achieved during insulin infusion (Fig. 1) was far higher than that in either fed or refeed animals. At concentrations of insulin similar to or slightly higher than those in fed or refeed rats, no effect of insulin or glucose infusion on protein synthesis was apparent (Tables 2 and 3).

Table 5 shows the results of an experiment designed to ascertain if insulin had any effect on protein synthesis outside the range of concentrations found in fed and postabsorptive animals. When fed rats were given infusions of insulin, at a rate estimated to be high enough to raise the concentration in plasma to 2.5 munits/ml, there was a large decrease in plasma glucose. However, protein synthesis was not altered. Similarly, when guinea-pig anti-insulin serum was infused there was an appreciable increase in plasma glucose. Although we were not able to measure the plasma insulin in the

presence of the antibody, the increase in plasma glucose is indicative of a decrease in insulin. Again this change did not have any effect on the rate of protein synthesis.

We have reported previously that injection of the flooding dose of [ $^3\text{H}$ ]phenylalanine results in an immediate rise in plasma insulin concentration, followed by a very rapid fall (McNurlan *et al.*, 1982). The results in Table 6 show that this did not affect the validity of our measurements of insulin and glucose in plasma obtained from rats 10 min after phenylalanine injection. In Expt. 4, additional animals from three of the groups were killed without receiving the injection of phenylalanine. Table 6 shows that the concentrations of insulin and glucose in plasma of these rats were the same as those from the phenylalanine-injected animals.

### Discussion

When rats are deprived of food there is a fall in the rate of muscle protein synthesis, which can be detected within a few hours (Garlick *et al.*, 1973) and becomes more pronounced as starvation progresses (Garlick *et al.*, 1975; Millward *et al.*, 1976). The results in Table 4 show that only 10–12 h after food withdrawal the rate of synthesis per g of RNA (i.e. per ribosome; Henshaw *et al.*, 1971; Millward *et*

Table 5. *Effects of increased insulin in fed rats and decreased insulin in postabsorptive rats*

Food was removed from fed rats immediately before infusion of insulin (4 units/h, 0.48 ml/h) or diluent for 30 min. Postabsorptive rats were deprived of food from 23:00h the previous day. Infusions of anti-insulin serum or non-immune serum (0.4 ml/h) were given for 1 h, after a priming dose of 0.1 ml. In all cases protein synthesis was measured during the last 10 min of infusion. Neither insulin nor antibody resulted in statistically significant changes in  $k_p$  or synthesis per g of RNA. Plasma glucose was significantly ( $P < 0.001$ ) decreased by insulin infusion and increased by antibody infusion.

Treatment	Plasma insulin ( $\mu$ units/ml)	Plasma glucose (mM)	$k_p$ (%/day)	Protein synthesis (g/day per g of RNA)
Fed (6)	22 $\pm$ 2	7.5 $\pm$ 0.4	18.0 $\pm$ 0.7	17.7 $\pm$ 0.6
Fed + insulin (6)	2500*	3.5 $\pm$ 0.2	17.1 $\pm$ 0.8	18.2 $\pm$ 0.5
Postabsorptive (6)	5 $\pm$ 2	5.9 $\pm$ 0.1	9.1 $\pm$ 0.7	10.0 $\pm$ 0.6
Postabsorptive + antiserum (6)	—	8.7 $\pm$ 0.2	9.6 $\pm$ 0.4	9.8 $\pm$ 0.5

\* Approximate value estimated by extrapolation from Table 2.

Table 6. *Effect of injection of 150  $\mu$ mol of [ $^3\text{H}$ ]phenylalanine on plasma insulin ( $\mu$ units/ml) and glucose (mM)*

All rats were postabsorptive. Glucose infusions were given for 30 min at a rate of 0.48 g/h and insulin infusions were given for 30 min at a rate of 37 munits/h. Phenylalanine-injected rats were given 150  $\mu$ mol of [ $^3\text{H}$ ]phenylalanine 10 min before death as described in the Methods section. Those not injected were killed after appropriate infusions. There were no statistically significant effects of phenylalanine injection on either insulin or glucose concentrations.

	Not injected		Phenylalanine-injected	
	Insulin	Glucose	Insulin	Glucose
Control	8.5 $\pm$ 1.5 (4)	6.7 $\pm$ 0.1	8.4 $\pm$ 1.0 (6)	6.9 $\pm$ 0.4
Glucose infusion	47.1 $\pm$ 2.2 (4)	14.6 $\pm$ 0.2	41.4 $\pm$ 4.1 (6)	14.0 $\pm$ 0.3
Insulin infusion	17.1 $\pm$ 6.7 (4)	5.3 $\pm$ 0.3	19.4 $\pm$ 1.4 (5)	5.5 $\pm$ 0.4

*al.*, 1973) had fallen from 17 to 10–11g of protein/day per g of RNA, although there was only a small decrease in the amount of RNA. The additional fall in the fractional rate of synthesis with longer periods of starvation appears to result mainly from a loss of RNA, since there is no further decline in synthesis per g of RNA (Garlick *et al.*, 1975; Millward *et al.*, 1976). Starvation is also accompanied by a fall in the concentration of insulin in the plasma. Furthermore, the changes in protein synthesis brought about by diabetes are quite similar to those resulting from starvation. In rats made diabetic with streptozotocin or alloxan, rates of synthesis per g of RNA fall to the same low value (9–11g of protein/day per g of RNA; M. Fern & P. J. Garlick, unpublished work) as those during starvation, when measured under the same conditions. The present investigation was therefore undertaken to find out whether higher rates of synthesis could be restored by replacement of insulin in starved rats, and to what extent the effects of food intake could be attributed to changes in the circulating concentration of this hormone. A probable role for insulin in the response to food intake has been deduced from experiments *in vitro* (e.g. Li *et al.*, 1979; Goldberg, 1979), but experiments *in vivo* are needed to clarify whether insulin can act alone, or in concert with other substances such as amino acids, glucose or other hormones, which also change in concentration after feeding.

Infusion of insulin rapidly resulted in an increase in protein synthesis in postabsorptive, but not in fed, rats. This is similar to the response that has been noted in experiments *in vitro*. In the perfused hemicorpus from fed rats Jefferson *et al.* (1974) showed that protein synthesis was unaffected by insulin during the first hour of perfusion. Subsequently there was a decrease in synthesis in the absence of insulin, presumably as that present in the muscle was lost, since it could be prevented by the addition of insulin to the medium. In perfused hemicorpus from starved rats, addition of insulin caused an increase in the rate of synthesis within the first hour, to a value similar to that in fed rats (Li *et al.*, 1979; Preedy *et al.*, 1979). Similarly, in incubated muscles, insulin stimulated protein synthesis, but the effect was more pronounced in those from starved than from fed rats (Fulks *et al.*, 1975; Frayn & Maycock, 1979).

In our experiments *in vivo* there was a delay of 10min before any effect of insulin on protein synthesis could be observed. This contrasts with the effect on plasma glucose, which had decreased significantly ( $P < 0.001$ ) by this time. After this there was an increase in synthesis, which persisted up to at least 1h. There may have been a peak at about 35–45min and a decline at 50–60min, but the changes between 10 and 60min were not great

enough to achieve statistical significance. In any case, such elevated rates of synthesis are unlikely to persist indefinitely. This is because in the absence of dietary protein intake the increase in synthesis must have occurred at the expense of the free amino acid pool or the protein of some other tissue.

These measurements of the time course involved concentrations of insulin that were somewhat higher than the normal physiological range. Most experiments *in vitro* have used still higher values, sometimes as high as 0.1 unit/ml (Fulks *et al.*, 1975). However, both Jefferson *et al.* (1977) and Frayn & Maycock (1979) have observed effects of the hormone when added at only 100  $\mu$ units/ml, which they estimated to be close to the value in fed animals. *In vivo* we were able to show that concentrations of 70  $\mu$ units/ml caused a significant increase in synthesis, but 40  $\mu$ units/ml or lower seemed to have no effect (Table 2). This latter point was important to establish, because in these experiments the concentration of insulin in fed rats was less than 40  $\mu$ units/ml (Table 4).

The infusions of glucose were performed to eliminate three potential problems in the interpretation of the insulin infusions. Firstly, it was possible that the hypoglycaemia during insulin infusion would interfere with the action of the hormone. In the glucose infusions the rats were hyperglycaemic, but still there was no effect on protein synthesis. This is consistent with the report by Jefferson *et al.* (1977) that omission of glucose from the medium of the perfused hemicorpus did not affect muscle protein synthesis. Secondly, there was a possibility that the source of the insulin might be important, since we infused pig insulin. Also, the assay that we used to measure the hormone in rat plasma was standardized against human insulin, and may not have given comparable values for endogenously produced rat insulin and infused pig insulin. This problem was overcome with glucose infusions, since the insulin was all endogenously produced, making it possible to compare values obtained in postabsorptive and fed rats with the elevated values induced by glucose. Thirdly, it was possible that, whereas the effect of insulin infusion was close to maximal at 20–30min at the high concentration used in Table 1, it could take longer to exert its effect at lower concentrations. However, even when the glucose infusion was prolonged for 1h it did not stimulate protein synthesis.

The infusion of anti-insulin serum represents an attempt to define further the range of concentrations over which insulin exerts its effect on protein synthesis in postabsorptive rats. Cost limited the length of infusion of antibody, and it is not certain that 1h would have been sufficient for any further decrease in synthesis to develop. Jefferson *et al.* (1977) and Li *et al.* (1979) have shown that at least

1 h of perfusion of hemicorpuses from fed rats in the absence of insulin (even in the presence of antibody) is required before the effect of the insulin present before perfusion disappears. We cannot say whether this same time period would apply to postabsorptive rats *in vivo*. However, from the fact that protein synthesis per g of RNA in diabetic rats (M. Fern & P. J. Garlick, unpublished work; see above) is the same as that in postabsorptive rats, it is unlikely that insulin has any measurable effect within the range 0–10  $\mu$ units/ml.

It therefore appears, from the failure to detect any effect of insulin on protein synthesis at less than 40  $\mu$ units/ml, that the hormone acts over the narrow range 40–70  $\mu$ units/ml. However, statistically it would not be possible to rule out the possibility of a linear response over the entire range, so until many more measurements are made this conclusion must remain tentative. The response of glucose metabolism to insulin is much more clear. Changes in plasma glucose concentration occurred over the entire range of insulin concentration, including those below the postabsorptive values achieved with antiserum infusion.

Measurements of the rates of synthesis in fed and refed rats show quite clearly that these were much higher than those in postabsorptive animals with similar insulin concentrations. Although both fed and refed rats had lower concentrations of insulin than that required to stimulate synthesis in the absence of food, additional insulin did not increase synthesis further (Table 4), but it did decrease the plasma glucose. Nevertheless, it is difficult to conclude that the changes in insulin after feeding are not related to the increase in protein synthesis. The discrepancy in concentration is about a factor of 2, which could result from the presence of other factors or to insulin resistance. Other hormones are known to be responsive to feeding. For example, we have shown that glucagon can inhibit protein synthesis in muscle, both in the perfused hemicorpus (Preedy *et al.*, 1980) and *in vivo* (Preedy & Garlick, 1982). Similarly it has been suggested that high concentrations of corticosterone cause insulin resistance in muscle of diabetic rats (Odedra *et al.*, 1982). However, postabsorptive rats are not so insulin-resistant as diabetics, which we have found to require very large doses of insulin to stimulate protein synthesis in muscle (i.e. 4 units given as an intraperitoneal injection 1–2 h before measurement of protein synthesis; M. Fern & P. J. Garlick, unpublished work). Amino acid concentrations might also be important. Studies with incubated and perfused muscle have shown that amino acids can increase the rate of protein synthesis and that this response can be attributed entirely to the branched-chain amino acids, particularly leucine (Fulks *et al.*, 1975; Li & Jefferson, 1978). However, we have not

been able to reproduce this effect when leucine was injected into fed, starved or protein-deprived rats *in vivo* (McNurlan *et al.*, 1982). Also, concentrations of branched-chain amino acids in plasma do not correlate with the measured rate of synthesis (Millward *et al.*, 1976). It is therefore unlikely that a substantial part of the response to feeding can be attributed to changes in free amino acids, but we cannot rule out the possibility that they act cooperatively with insulin. Although insulin has no action on its own at concentrations below 40  $\mu$ units/ml, it may act permissively in allowing nutrients such as amino acids in the blood to stimulate protein synthesis.

Finally, although synthesis per g of RNA in the rats given the higher doses of insulin came quite close to that in refed rats, rates in the rats fed *ad libitum* were higher still. Within Expt. 2 these differences were not statistically significant, but, taken overall, the two fed groups had higher rates than any of the insulin-treated or the refed groups. This suggests that the effect of feeding may contain a component which takes more than 0.5–1 h to develop. Since the effect of insulin appeared to be maximal within this period, this again suggests that the response of protein synthesis to feeding involves more than just the effects of changing insulin concentrations.

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